Public Hearing (12/15/10)
CEC – Recycled Water
Deadline: 1/10/11 by 12 noon

Subject: Comment Letter (Addendum to 12-03-10) - CEC Monitoring for Recycled Water

Jeanine Townsend, Clerk to The Board
State Water Resources Control Board
1001 I Street, 24th Floor
Sacramento, CA 95814

RECEIVED
DEC 7 2010
SWRCB EXECUTIVE

Public Comment Addendum to Part I (emailed 12-03-10)

Re: Public Comment Addendum to our initial Comment emailed to Jeanine Townsend on Dec. 3, 2010 re: Dec. 15, 2010 Public Comment session in Sacramento
From: John M. Ackerman, M.D.

Dear Panel Chairman and other Panel Members:

Please read and digest this supplemental comment prior to The Dec. 15, 2010 meeting in Sacramento.

This public comment forum is designed to address CEC's in recycled water. The recent customary Federal use of the definition of CEC's regarding wastewater is: Contaminants of Emerging Concern, not Chemicals of Emerging Concern. Some people supplying public comment for the Dec. 15 public comment might , both in writing and verbally, be responding to the broader Federal definition of CEC's. We hope that this hearing will not dismiss the comments of those people.
First Questions for Board: There are several terms that have been used for CECs. This sets up confusion. The topic seems to be constantly shifting. Please define the technical, policy and regulatory difference in the approach to the following terms, all of which seem to have been variously used in the current discussion by your Board: 1) constituents of emerging concern, 2) contaminants of emerging concern, and 3) chemicals of emerging concern. Which is the least restrictive and again which is the most restrictive and why is such the case?

The Blue Ribbon Panel on Contaminants of Emerging Concern (CEC Panel) as relate to recycled water usage has presented its final draft document and thus the State Water Resources Board is seeking public comment. Although that Panel was not charged with a review of antibiotic resistance (that effort and topic were originally reserved for a second expert panel that was never established) the CEC Panel, while admitting that it was not expert in the subject, sought, nonetheless, to comment. Unfortunately, because the CEC Panel chose to speak to the issue of antibiotic resistance, the Board may feel obliged to give a certain credence to these statements. This gratuitous move was potentially dangerous because of the critical nature of the subject matter and the inexperience with the subject as found within the CEC Panel. It was dangerous for a number of reasons, but mainly because neither the Board nor its staff are expert in the topic of antibiotic resistance as generated by sewage processing, but are inclined to nonetheless generate policy.

Question for the Board: Is a second panel expected for review of antibiotic resistance, as originally envisioned? If yes, when? If not, why? The Panel of CEC's was not expert in the area of antibiotic resistance and indicated so. If the Board chooses to use determinations from the work by the CEC Panel on antibiotic resistance, how will it justify that stance? Where is the objective scientific evidence within the CEC Panel's work to make determinations on antibiotic resistance? The CEC Panel itself indicates that more information is needed on the CECs. However, we now need to know what the Panel means by CECs (see the first question)?

LaPara, et al (see below) produced a study discussing the relationship between the viability of antibiotic resistant genes (ARG's), as generated by sewer plants. The paper by LaPara discussed the potential risk to public health plus the direct relationship between survival of genetic material and the temperatures at which the sludge was processed. He noted that when sludge was processed at temperatures typically used by the majority of current sewer plants (mesophilic---body temperature), the resistant genes survive to reach the environment.

Question for the Board: What are the immediate and then long-term plans by the Board for curbing the generation of ARGs and antibiotic resistant microbes in general? Please read the 2 questions in the next paragraph.
LaPara's current work mirrors and expands on the research done in the early 1980's by US/EPA. Thus, critical questions must be asked of the overall regulatory community: 1.) Why have the designs of sewer plants and their operation not changed in response to this information on antibiotic resistance and 2.) Why do the standards not reflect the existence, hence risk from ARG's? These are long-known facts and yet the regulatory community for the last 30 years has not responded while the rates of antibiotic resistant infections have been increasing. Why is this?

Questions for the Board: In addition to the questions in the above paragraph, what are the immediate and then long-term plans by the Board to correct the release of antibiotic resistant microbes and their genetic material?

It is important to answer this question because of the increasing potential contagious danger of Staph aureus, E. coli and Klebsiella all now causing serious diseases arising in the midst of our communities. We are being overrun by the adverse effects of antibiotic resistant pathogens. At the same time, our drug armamentarium is diminishing because of advancing resistance. To add insult to injury, the pharmaceutical industry shows disinterest in developing new antibiotics. There is nothing really new with this picture. It is a picture that is not obscure. Given this scenario and the elapsed time, where is, and has been, the regulatory community that is presumed to be operating at taxpayer expense to protect the public health?

First, it will be important to trace, briefly, through the processing of sewage and thus touch upon two of the three byproducts coming out of sewage processing. This is done because there is a direct relationship between recycled water quality and how sewer plants are designed and run. As LaPara notes, the vast majority of sewer plants use mesophilic temperatures and run the solids completely through the plant taking digested solids off at the end of the process. This complete transit through the plant offers numerous time opportunities to enhance the multiplication of resistant organisms and their resistant genes that vastly increase the rate and level of antibiotic resistance (other processes that obviate this will be discussed below).

LaPara notes that this current type of mesophilic processing is responsible for the release of excess numbers of antibiotic resistant genes (ARG's). For the reader, unfamiliar with ARG's, these are free-floating gene fragments that can be taken in by other non-contaminated bacteria and coupled to their genetic structure thus also rendering the non-contaminated organisms resistant to antibiotics. These genes are so small that they easily pass through the typical filters used in water treatment and, because they are not "alive" in the sense of a complete cell, are unaffected by chlorine at levels typically used in water treatment (for sewage or drinking). Thus, these ARG's sail right through the plants into the environment. ARG's are now also found in drinking water. When they are taken into the human intestine, the domestic internal bacteria that are so necessary for human survival are contaminated and turned into lending libraries of antibiotic resistance. These lending libraries remain functional for years. Later, incoming pathogens may borrow this information rendering them resistant to antibiotics and consequently, the infection may be unstoppable.

Because this is a discussion about sewer plants, it includes a sub-discourse on how the facilities generate and then release antibiotic resistant pathogens into the environment. The release of antibiotic resistant pathogens, beyond debate, is a documented fact known by the US/EPA for at least three decades. Yet, that agency has done nothing with that research information. Of particular interest to this discussion is the topic of recycled water, especially as produced under criteria developed by the State of California. As documented in the first part of our public comment emailed 12-3-10 to the secretary of the State Water Resources Control Board, Edo McGowan, Ph.D. tested the finished disinfected recycled water meeting all applicable state requirements and cultured a range of multi-antibiotic resistant serious pathogens from two sewer districts that produce recycled water under Title 22.

Questions for the Board: Allowing for the fact that the study by the US/EPA from its Wastewater Research Division, Municipal Environmental Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio, was published in 1982 and the State of California was subsequently designated by the EPA to oversee water quality as related to the Clean Water Act, how is it that 30 years have elapsed with the knowledge that sewer plants generate and release of antibiotic resistant organisms and there are no California Standards dealing with this potential public health risk? The State of California is well aware of McGowan's research results. He has suggested that the state repeat his work. Thus far, the state continues to ignore the situation and has essentially turned a blind eye to the underlying public health risks.

We again come forward to the State Water Resources Control Board regarding the public comment session in Sacramento on Dec. 15, 2010 where our words become part of the public record. For posterity and legal purposes, all of our previous comments on recycled water as submitted to the SWRCB or its Regional boards by Edo McGowan, Ph.D. and John Ackerman, M.D. are herein incorporated by reference.

There is a direct connection between how solids (both in suspension and in solution) are processed in a typical wastewater sewer plant and how that affects the sanitization of recycled water and the discharged effluent. Sludge (biosolids) are the solid materials extracted from wastewater. The cleaner the released effluent and recycled water, the dirtier the biosolids which concentrate pollutants. Paradoxically, the longer the sludge is processed the higher the counts of multi-antibiotic resistant organisms in all 3 byproducts (effluent, recycled water and biosolids). The explanation for this paradox is that more time in processing solids facilitates more transfer of resistant genes to the normal bacteria positioned in the treatment plants to digest waste material. This, consequently, creates many more multi-antibiotic resistant organisms and their genes to be present in all 3 byproducts of the treatment process.

There are new cutting-edge wastewater treatment technologies that remove the solids from the raw water influent just prior to entering the plant removing a large portion of the entering materials including antibiotic resistant pathogens. With this newer technology, these removed solids are dewatered sufficiently to maintain auto-combustion when injected into a fluid bed reactor. The heat of the reaction destroys many of the undesirables that accompany sewage and the remaining ash can be recycled. The process mainly converts the solids to a usable syngas in the range of C3 through C8. This is a gas that has slightly higher BTU value than natural gas. The efficiency of this technology is about 85%, i.e., 15% of the available energy in the extracted solids is needed to maintain combustion and the remaining 85% comes off as usable and/or marketable energy. There are whole towns in Europe that use similar technology to heat homes, thus offsetting major energy purchases. The overhead expenses of running such cutting edge technology are greatly reduced. The considerable savings can be transferred to other budgets.

Questions for the Board: Please detail the Board's efforts in reviewing this new technology. Has the Board actually reviewed this new technology If so, please indicate which technology?

Let us now discuss the typical current technology, a design that has not appreciably changed from that available in the first quarter of the 20th Century. Historically, the main function of a sewer plant was to reduce the stench of sewage. As time passed, the main purpose of the plant was to consume as much material as possible that contributed to the biological oxygen demand that caused eutrophication of water bodies. This early era of the 20th Century was a time predating the organic chemical revolution that followed WW2, This was also a time that predated mass industrial discharges to sewers and a time predating the addition and acknowledgement of contaminants of emerging concern (CECs). The sewer plants were never designed for these later events. But, perhaps more importantly (since sewer plants as currently designed rely on the work of microbes), these old designs also predated the antibiotic revolution which predated the development of antibiotic resistant microbes and science's knowledge of gene transfer. This older technology is still found serving many of the cities in the U.S. To the untutored, this antiquated technology still "appears" to function, albeit often out of compliance with provisions of the Clean Water Act.

As a consequence of complying in 2010 with antiquated Federal Standards, the California facilities pump out untold levels of antibiotic resistant bacteria some of which have reached considerable notoriety as very serious Superbugs. Adding to the seriousness of this picture is the fact that, according to the CDC, a single serious pathogen (MRSA) out of the myriad of serious antibiotic resistant pathogens released by sewer plants now kills more Americans than AIDS through infections that cannot be stopped (see reference in our original comment to the Board dated 12-03-10).. Sewer plants are one of the principal generators of antibiotic resistant microbes. This, again is not new information, but where are the regulators in all of this, where is the push to require industry to develop new designs?

Questions for the Board: Please demonstrate the levels of effort in evaluating new designs for sewer plants that would obviate the issues surrounding antibiotic resistance.

"How does the current sewer plant work?" warrants discussion. A sewer plant is typically set up to screen out really big materials from the incoming wastewater. The remaining solids then go into a series of settling tanks (clarifiers) where the remaining solids are differentially settled out, gathered and concentrated. These solids are then moved (pumped) to a series of digesters where (through massive and highly concentrated bacterial action) the solids are digested (broken down and partially consumed by microbes). If one did an analysis of an input to output on these solids, one would find that through bacterial digestion (and the high multiplication of microbes in the process) there will actually be a greater mass of solids being removed from the sewer plant than initially came into the plant. In the process of digestion, the microbes break down many solids and convert them into solution. However, Sewer plants of current design do not effectively deal with materials in solution. These materials in solution will tend to go right through sewer plants into the environment along with the increasing levels of pharmaceuticals in the nation's drinking water. A major portion of this nation's drinking water is extracted from rivers below an up-river sewer outfall. Keep this picture in mind as we continue to discuss how the sewage processing works.

The above mentioned LaPara paper noted that an increased temperature during digestion of solid material would greatly reduce the number of surviving antibiotic resistant genes (ARG's). He noted that this reduction in ARG's occurred at temperatures of 130F. The question remains, is this temperature sufficient to destroy all the genetic information? In reviewing work on heat killing of pathogens, one is reminded of Griffith's work (see below). In Griffith's work, there were times that the experiments reached 176 F and the microbes still survived. Griffith's experiments were characteristically run above temperatures discussed by LaPara and included the gene transfers from heat killed pathogens to and into benign bacteria. In this single step of gene transfer, benign bacteria were transformed into lethal pathogens.

Another confounder is the issue of encysted microbes which are well protected from both heat and disinfectants. Further, there are spores that are protected and can withstand considerably higher temperatures. Also, as seen below by Moce-Llivina, et al there was survival of pathogens in sludge as high as 176F which included spores of sulfite-reducing clostridia.

We believe that LaPara's work is certainly a step in the right direction. It importantly documents that the naked genes are a potentially serious public health issue, that current sewer plant designs fail to eliminate them and heating could be an interim strategy for their reduction. But this should not be accepted as the end-all, be-all, which I'm sure LaPara is not arguing. However, the industry may grasp at this potential new step and say,"See, here the problem is solved." Heating at LaPara's suggested temperatures may not effectively deal with resistant microbes that require high-level disinfection. To the extent that industry dumps microbial materials into the sewer from such sources as high-temperature designer microbial production systems that are now becoming more frequently used by industry, there will be an up-shift in those organisms that are able to handle higher temperatures. When they are dumped into sewers they may live quite comfortably in systems well above temperatures discussed by LaPara and thus be able to accept pathogenic and resistant genetic information, maintain it, and pass it on.

Aside from the need to destroy the genetic information, there are many constituents contained in sewage concentrated into sludge that are damaging to the environment and these are essentially not impacted by the elevated temperature. The halogenated organic materials such as brominated flame retardants remain unaffected. These materials (in the effluent and/or recycled water also find their way into the environment by passing through fairly tight membrane systems. One paper noted that RO was only able to catch 95% of the flame retardants.

Ozonation and advanced oxidation technologies to remove endocrine disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs) in water effluents
Santiago Esplugasa
<http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6TGF-4P9625P-6&_user=10&_coverDate=11/19/2007&_rdoc=1&_fmt=high&_orig=search&_origin=search&_sort=d&_docanchor=&view=c&_searchStrId=1569797723&_rerun> ,
<http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6TGF-4P9625P-6&_user=10&_coverDate=11/19/2007&_rdoc=1&_fmt=high&_orig=search&_origin=search&_sort=d&_docanchor=&view=c&_searchStrId=1569797723&_rerun> , <mailto:esplugas@angel.qui.ub.es>

, Daniele M. Bilab
<http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6TGF-4P9625P-6&_user=10&_coverDate=11/19/2007&_rdoc=1&_fmt=high&_orig=search&_origin=search&_sort=d&_docanchor=&view=c&_searchStrId=1569797723&_rerun> , Luiz Gustavo T. Krausec
<http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6TGF-4P9625P-6&_user=10&_coverDate=11/19/2007&_rdoc=1&_fmt=high&_orig=search&_origin=search&_sort=d&_docanchor=&view=c&_searchStrId=1569797723&_rerun> , 1
<http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6TGF-4P9625P-6&_user=10&_coverDate=11/19/2007&_rdoc=1&_fmt=high&_orig=search&_origin=search&_sort=d&_docanchor=&view=c&_searchStrId=1569797723&_rerun> and Márcia Dezottic
<http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6TGF-4P9625P-6&_user=10&_coverDate=11/19/2007&_rdoc=1&_fmt=high&_orig=search&_origin=search&_sort=d&_docanchor=&view=c&_searchStrId=1569797723&_rerun> , 1
<http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6TGF-4P9625P-6&_user=10&_coverDate=11/19/2007&_rdoc=1&_fmt=high&_orig=search&_origin=search&_sort=d&_docanchor=&view=c&_searchStrId=1569797723&_rerun> ,

US EPA---Treating Contaminants of Emerging Concern
A Literature Review Database
August 2010

For treatment of selected CECs in full-scale treatment systems that included RO, the average reported removal efficiencies are listed in Table 9. RO effectiveness varied by type of water treated. For treated effluent, the database includes removal efficiencies for 14 of the 16 CECs. The average removal efficiencies for treated effluent ranged from 81% for sulfamethoxazole to 100% for iopromide, triclosan, and naproxen.

Assuming some large output loads, Hyperion, for example, at 450 mgd or smaller systems such as Oxnard at 30 mgd, between 5% and 20% being missed is not a small volume. These figures are assuming that the membranes function as specified. However, it is known that as these membranes age, the % of rejected contaminant falls off.

Questions for the Board: As noted, the scientific literature on exclusion of contaminants by reverse osmosis (RO) notes that it may be less than 100% effective and in some cases significantly less, ranging up to nearly 20% missed. How does the Board plan to deal with these levels, especially for mixing recycled water with ground water, its use on crops consumed raw or on agricultural lands, pasture lands for animals to be marketed for consumption and municipality grass including golf courses ?

In our first comment of 12-03-10 we strongly recommended a moratorium on these uses of recycled water until the CDC completes their research that the Panel has advised. Also, please refer to the Rep. Lois Capps attachment in our comment of 12-03-10 that includes detailed recommendations how to upgrade the design of wastewater treatment plants and research that needs to be considered.
So, for a moment, assume we view the typical plant and merely run up the temp. What else comes through with the sludge unaffected by a mere rise in temperature and what are the economic considerations? We have essentially the same level of heavy metals coming through (as the temp should not impact those). We have most of the CEC's (all contaminants including biological and not just chemicals of emerging concern) still coming through and concentrating in the sludge. However, as noted above, some of the solids are converted to solutions. Since sewer plants do a poor job with materials in solution, many of these in-solution pollutants are partitioned

into the recycled water. In fact, the work of Chad Kinney documents that recycled water carriespharmaceuticals. Some of those bioaccumulate in the soils. Many other CEC's also come through in recycled water and these, like pharmaceuticals, can bioaccumulate in crops.

Let's look at sludge a bit more and suggest that current land application is not a wholly sound approach. The objective here is to show the public health and environmental costs of remaining with the current designs in which sewage sludge is digested and thus retained within the sewer plant, hence enhancing the generation of antibiotic resistance. It is important for the decision-maker and public record to understand these costs as compared to costs associated with the up-front removal of the solids, hence the destruction of detractors. In fact, the main sewer plant in St Paul did an extensive economic analysis of using sewage sludge as a fuel source and compared the costs and benefits to land application. Their analysis demonstrated it was economically more advantageous to use the sludge as a fuel. Let me now run through a brief trip of delivering sludge from the WWTP to the farm. We will use Orange Co-LA sewer sludge as the example, starting at Hyperion in Long Beach and trucking the sludge to Bakersfield.

Using Hyperion data, the tonnage of sewage sludge produced by the plant is approximately 650 tons/day. It must be transported by truck over the Tehapies, something like a climb of in excess of 4,000 feet in large trucks that average 6 to 8 mpg at $ 3.25/gallon of diesel over approximately 120 miles up and the same distance back. Thus 240 miles at an average of 7 mpg uses 34 gallons or roughly $110/truck in fuel alone. Assuming a 25 ton load, that's 26 truck loads/day. There are, of course, the diesel engine combustion byproducts that accrue to this haulage. But it also costs to operate those trucks in wear, maintenance, and depreciation, so absent fuel, the machinery cost is about 90 cents/mile and that is highly conservative because it does not consider the opportunity costs from lost income from a parallel investment of the truck's initial cost and it does not consider the cost of the driver, the insurance, the license, etc. Then we have lost rubber going into the atmosphere as latex particulates which are highly reactive allergens with lung tissues. These particulates are the size that will reach the deepest recesses of the lung tissue. The average car, for example, sheds about 10# of rubber a year and thus the trucks proportionately more. On top of that we have the occasional accident, the spillage and thus the environmental contamination and response costs to public services that must be brought into the equation.

We have not delivered the material yet and there are some well documented environmental and public health issues associated with land applied sewage sludge. First, according to EPA's Region IX, each ton of land applied sewage sludge will off-gas an average of 3,000 cu ft of CH_4, a serious greenhouse climate changing gas. Then there is the addition of the CECs to the environment and their movement into the atmosphere and hydrosphere. These materials also travel in storm water thus reaching riparian systems, especially an issue in the Central Valley which is a sink and where this material accumulates presenting a risk to the underlying ground water.This then is a thumb nail sketch of financial and environmental issues associated with sewage sludge.

Questions for the Board: Because of the provisions of AB 32 relating to climate change, what is the Board doing to coordinate on the greenhouse off gasing from land applied sewage sludge and the types of sewer plant designs that would obviate the need to land apply?

However, If we take the solids off the incoming waste-stream before they enter the plant, a series of positive events accrue. First, the solids can be easily concentrated and converted into a highly usable fuel source. The conversion would destroy many already resistant pathogens and their genetic material as well as pharmaceuticals. Valuable metals can be retained from the ash. The subsequent use of high pressure micro ozone would be able to disrupt any biological and chemical bonds remaining in solution at the end of the treatment process. This last step would go a long way toward assuring that ARG's are completely eliminated and that bonds within other contaminants of emerging concern are broken. Use of high pressure ozone at the end stage of the plant treatment would be more effective than the use of special filters as alluded to in our comment of 12-03-10.

With ozone, oxidation of organic matter and precipitation of metals further improve the water quality allowing its reuse. Over 100 viruses excreted in human feces have been reported in contaminated water any of which could cause a waterborne disease. Intestinal parasites, enteric bacteria and viruses are organisms of the greatest concern for human health (EPA, 1986). Therefore, discharging wastewater without proper disinfection poses a direct potential threat to human health.

Ozone is a powerful oxidant that destroys micro-organisms through an irreversible physicochemical action. Ozone does not have to penetrate the body of the micro-organism to inactivate it. On the contrary, the action of ozone is instantaneous and irreversible, first on the micro-organisms' protective wall and then on the semi-permeable membrane (Finch, 1999). Such action modifies the chemical structure of the micro-organism through a coagulation effect that causes a hindrance on any exchange of product with the outside. As a result, the microorganisms "suffocate" to death or to inactivation. The protective wall and the semi-permeable membrane are composed of molecules that are very rich in electron sites (Gaudy, 1980).
This favors a very selective, and therefore efficient, action by ozone. These physico-chemical reactions present extremely rapid kinetics (the corresponding half-life time of ozone is in the order of a second).

Unlike ozone, UV rays must penetrate the cell in order to be absorbed by the intracellular nucleic acids. These acids are the sites where the UV rays can kill or inactivate the microorganism (EPA, 1986). Interestingly, in a discussion with Amy Pruden, LaPara's coauthor, it was mentioned that in inspecting bacteria killed with UV, the critical genetic information conferring antibiotic resistance was not damaged. As we have seen above, genetic information that is conserved within dead bacteria is available to live bacteria (Griffith's work).In addition, UV rays do not necessarily cause irreparable damage; it has been proven that with UV there is always a non-negligible degree of reactivation. This is very important when required to reach inactivation levels of 3 log (99.9 %) or more. For certain micro-organisms such as Cryptosporidium Parvum and Giardia, extremely high UV dosages are necessary.It is also important to note that ozone does not propagate in straight lines but diffuses in all directions, even in-side flocs where it can reach hidden micro-organisms, whereas UV ray dispersion is negligible. It has even been demonstrated that the micro-organisms seek shelter from UV rays inside the flocs. Meckes back in the 1980s, in doing the EPA study, noted that UV actually enhanced antibiotic resistance. Before UV use on strains exhibiting resistance represented 40% of all isolates and after UV they represented 70%.

Questions for the Board: As noted above, UV is not fully effective in disinfection. Additionally, RO has been shown to be significantly less effective than 100% effective. As noted by LaPara, sewer plants generate ARG's and as documented by Harwood, Title 22 recycled water is not, by any stretch of the imagination, free of pathogens. The current Standards fail to ascertain the presence of these pathogens.

What steps will the Board take to overcome these deficits and thus demonstrate that it is, in fact, protecting public health? When will the Board initiate the research studies for developing new Standards. What will the Board do in the interim, absent effective Standards? Will the Board embrace the moratorium that we recommended above re: certain future uses of recycled water until the proper research has been completed?

Under H&SC 5410, et seq, the terms nuisance, contaminant, and pollutant are discussed. Please indicate whether or not antibiotic resistant genes and antibiotic resistant microbes would be precluded from consideration of these terms and sections as well as the complementary sections in the Water Code defining the same terms: nuisance, contaminant, pollutant? Please indicate what the criteria are for either including a constituent or excluding a constituent such as antibiotic resistant genes, from these defined terms.

Citations and abstracts of interest:

Gaudy & Gaudy . **Microbiology for Environmental Scientists and Engineers.** McGraw-Hill series in water resources and environmental engineering, 1980. U.S. Environmental Protection Agency (1986) "Design Manual - Municipal Wastewater Disinfection", EPA/625/1-86/021

Downie, A.W. **Pneumococcal Transformation-A Backward View Fourth Griffith Memorial Lecture,** J Genl Microbio (73)1:1-11(1972); http://mic.sgmjournals.org/cgi/reprint/73/1/1?view=long&pmid=4143929 <http://mic.sgmjournals.org/cgi/reprint/73/1/1?view=long&pmid=4143929>

Dawson confirmed Griffith's observation that transformation did not occur if the smooth type 111 suspension was heated above 80 "C (176 F).He further found that heat-killed preparations from old cultures were useless, and that freezing and thawing of previously effective preparations destroyed the transforming principle(Sia & Dawson, 1931). He suggested that the essential factor was destroyed by bacterial enzymes. Pure S.S.S. - that is, type-specific capsular polysaccharide from type 111 failed to transform rough forms derived from type 11. Later he found that even smooth type I1 could be transformed into type 111, apparently without the intermediary of rough forms (Dawson & Warbasse, 1931). The next step was taken by Alloway, also working in Avery's laboratory (Alloway, 1932, 1933). He found that transformation could be achieved by bacteria-free extracts done prepared from young cultures of virulent pneumococci. The sedimented bacteria were dissolved by sodium desoxycholate and heated at 60 "C (149 F) for 10 minutes. The extracts could be filtered through Berkefeld candles, and absorbed with charcoal, without losing activity. The transforming principle could be precipitated by 10 vol. of alcohol or acetone. The activity of the final preparation was such that 0.05 ml of extract (50 ml concentrated from 5 1 of culture) was sufficient to produce transformation of R forms in the presence of serum or serous fluid. These extracts contained specific polysaccharide in sufficient concentration to induce active immunity in mice. However, Alloway did not think that specific polysaccharide was the transforming principle and concluded that 'if S.S.S. is involved it is present in a different physical state, or in combination with some other substance which confers on it properties not found in the purified substance '.

Survival of bacterial indicator species and bacteriophages after thermal treatment of sludge and sewage.

Mocé-Llivina L .

Department of Microbiology, Faculty of Biology, University of Barcelona, Avenida Diagonal 645, 08028 Barcelona, Spain.
The inactivation of naturally occurring bacterial indicators and bacteriophages by thermal treatment of a dewatered sludge and raw sewage was studied. The sludge was heated at 80 degrees C, and the sewage was heated at 60 degrees C. In both cases phages were significantly more resistant to thermal inactivation than bacterial indicators, with the exception of spores of sulfite-reducing clostridia. Somatic coliphages and phages infecting Bacteroides fragilis were significantly more resistant than F-specific RNA phages. Similar trends were observed in sludge and sewage. The effects of thermal treatment on various phages belonging to the three groups mentioned above and on various enteroviruses added to sewage were also studied. The results revealed that the variability in the resistance of phages agreed with the data obtained with the naturally occurring populations and that the phages that were studied were more resistant to heat treatment than the enteroviruses that were studied. The phages survived significantly better than Salmonella choleraesuis, and the extents of inactivation indicated that naturally occurring bacteriophages can be used to monitor the inactivation of Escherichia coli and Salmone

Fighting Antibiotic-Resistant Bacteria by Treating Municipal Wastewater at Higher Temperatures, La Para, Timothy M.

ScienceDaily (Nov. 23, 2010) — New findings by civil engineering researchers in the University of Minnesota's College of Science and Engineering shows that treating municipal wastewater solids at higher temperatures may be an effective tool in the fight against antibiotic-resistant bacteria. Heating the solid waste to 130 degrees Fahrenheit (55 degrees Celsius) was particularly effective in eliminating the genes that confer antibiotic resistance. These genes are used by bacteria to become resistant to multiple antibiotics, which are then known as "superbacteria" or "superbugs."

The research paper was recently published in Environmental Science & Technology, a journal of the American Chemical Society and highlighted in the society's weekly magazine Chemical & Engineering News.
Antibiotics are used to treat numerous bacterial infections, but the ever-increasing presence of antibiotic-resistant bacteria has raised substantial concern about the future effectiveness of antibiotics.

"The current scientific paradigm is that antibiotic resistance is primarily caused by antibiotic use, which has led to initiatives to restrict antibiotic prescriptions and curtail antibiotic use in agriculture," said civil engineering associate professor Timothy LaPara, an expert in both wastewater treatment and microbiology who led the new University of Minnesota study. "Our research is one of the first studies that considers a different approach to thwarting the spread of antibiotic resistance by looking at the treatment of municipal wastewater solids."

Antibiotic resistant bacteria develop in the gastrointestinal tracts of people taking antibiotics. These bacteria are then shed during defecation, which is collected by the existing sewer infrastructure and passed through a municipal wastewater treatment facility. The majority of wastewater treatment plants incubate the solid waste, called sludge, in a "digester" that decomposes organic materials. Digesters are often operated at 95 to 98 degrees Fahrenheit (35 to 37 degrees Celsius). "Many digesters are operated at our body temperature, which is perfect for resistant bacteria to survive and maybe even grow," LaPara said. Lab research by LaPara and his graduate student David Diehl shows that anaerobic digestion of municipal wastewater solids at high temperatures (as high as 130 degrees Fahrenheit or 55 degrees Celsius) is capable of destroying up to 99.9 percent of various genes that confer resistance in bacteria. In contrast, conventional anaerobic digestion (operated at 95 to 98 degrees Fahrenheit or about 37 degrees Celsius) demonstrated only a slight ability to eliminate the same set of genes.

"Our latest research suggests that high temperature anaerobic digestion offers a novel approach to slow the proliferation of antibiotic resistance." LaPara said. "This new method could be used in combination with other actions, like limiting the use of antibiotics, to extend the lifespan of these precious drugs."
LaPara also pointed out that raising the temperature of anaerobic digestion at wastewater treatment plants is not cost-prohibitive because the digesting bacteria produce methane gas that can be used to heat the reactor.
The Minnesota Environmental and Natural Resources Trust Fund financially supported LaPara's recent research. LaPara has secured a grant from the National Science Foundation to continue his research examining other technologies to eliminate antibiotic-resistant bacteria in wastewater solids. http://www.sciencedaily.com/releases/2010/11/101122172135.htm

<http://www.sciencedaily.com/releases/2010/11/101122172135.htm>

>
Sci Total Environ. 2009 Jun 1;407(12):3702-6. Epub 2009 Mar 24.
Wastewater treatment contributes to selective increase of antibiotic resistance among Acinetobacter spp.
Zhang Y, Marrs CF, Simon C, Xi C.
Department of Environmental Health Sciences, University of Michigan, Ann Arbor, USA.
Abstract

The occurrence and spread of multi-drug resistant bacteria is a pressing public health problem. The emergence of bacterial resistance to antibiotics is common in areas where antibiotics are heavily used, and antibiotic-resistant bacteria also increasingly occur in aquatic environments. The purpose of the present study was to evaluate the impact of the wastewater treatment process on the prevalence of antibiotic resistance in Acinetobacter spp. in the wastewater and its receiving water. During two different events (high-temperature, high-flow, 31 degrees C; and low-temperature, low-flow, 8 degrees C), 366 strains of Acinetobacter spp. were isolated from five different sites, three in a wastewater treatment plant (raw influent, second effluent, and final effluent) and two in the receiving body (upstream and downstream of the treated wastewater discharge point). The antibiotic susceptibility phenotypes were determined by the disc-diffusion method for 8 antibiotics, amoxicillin/clavulanic acid (AMC), chloramphenicol (CHL), ciprofloxacin (CIP), colistin (CL), gentamicin (GM), rifampin (RA), sulfisoxazole (SU), and trimethoprim (TMP). The prevalence of antibiotic resistance in Acinetobacter isolates to AMC, CHL, RA, and multi-drug (three antibiotics or more) significantly increased ($p<0.01$) from the raw influent samples (AMC, 8.7%; CHL, 25.2%; RA, 63.1%; multi-drug, 33.0%) to the final effluent samples (AMC, 37.9%; CHL, 69.0%; RA, 84.5%; multi-drug, 72.4%), and was significantly higher ($p<0.05$) in the downstream samples (AMC, 25.8%; CHL, 48.4%; RA, 85.5%; multi-drug, 56.5%) than in the upstream samples (AMC, 9.5%; CHL, 27.0%; RA, 65.1%; multi-drug, 28.6%). These results suggest that wastewater treatment process contributes to the selective increase of antibiotic resistant bacteria and the occurrence of multi-drug resistant bacteria in aquatic environments.
PMID: 19321192 [PubMed - indexed for MEDLINE]

>
Most infections occur in immunocompromised individuals, and the strain *A. baumannii* is the second most commonly isolated nonfermenting bacteria in human specimens. *Acinetobacter* is frequently isolated in nosocomial infections and is especially prevalent in intensive care units, where both sporadic cases as well as epidemic and endemic occurrence is common. *A. baumannii* is a frequent cause of nosocomial pneumonia, especially of *late-onset* ventilator associated pneumonia. It can cause various other infections including skin and wound infections, bacteremia, and meningitis, but *A. lwoffi* is mostly responsible for the latter. *A. baumannii* can survive on the human skin or dry surfaces for weeks.
Since the start of the Iraq War, over 700 U.S. soldiers have been infected or colonized by *A. baumannii*. Four civilians undergoing treatment for serious illnesses at Walter Reed Army Medical Center in Washington, D.C., contracted *A. baumannii* infections and died. At Landstuhl Regional Medical Center, a U.S. military hospital in Germany, another civilian under treatment, a 63-year-old German woman, contracted the same strain of *A.*

baumannii infecting troops in the facility and also died. These infections appear to have been hospital acquired. [1] Based on genotyping of *A. baumannii* cultured from patients prior to the start of the Iraq War it is likely the soldiers contracted the infections while hospitalized for treatment in Europe.

[edit] Treatment

Acinetobacter species are innately resistant to many classes of antibiotics, including penicillin, chloramphenicol, and often aminoglycosides. Resistance to fluoroquinolones has been reported during therapy and this has also resulted in increased resistance to other drug classes mediated through active drug efflux. A dramatic increase in antibiotic resistance in *Acinetobacter* strains has been reported by the CDC and the carbapenems are recognised as the gold-standard and treatment of last resort.[4] Acinetobacter species are unusual in that they are sensitive to sulbactam; sulbactam is most commonly used to inhibit bacterial beta-lactamase, but this is an example of the antibacterial property of sulbactam itself.
[5]

In November, 2004, the CDC reported an increasing number of *A. baumannii* bloodstream infections in patients at military medical facilities in which service members injured in the Iraq/Kuwait region during Operation Iraqi Freedom (OIF) and in Afghanistan during Operation Enduring Freedom (OEF) were treated.[6] Most of these were multidrug-resistant. Among one set of isolates from Walter Reed Army Medical Center, 13 (35%) were susceptible to imipenem only, and two (4%) were resistant to all drugs tested. One antimicrobial agent, colistin (polymyxin E), has been used to treat infections with multidrug-resistant *A. baumannii*; however, antimicrobial susceptibility testing for colistin was not performed on isolates described in this report. Because *A. baumannii* can survive on dry surfaces for up to 20 days, they pose a high risk of spread and contamination in hospitals, potentially putting immune-compromised and other patients at risk for drug-resistant infections that are often fatal and, in general, expensive to treat.
http://www.ncbi.nlm.nih.gov/pubmed/19321192
>

ANTIBIOTIC RESISTANT BACTERIA AND THEIR GENES IN RECYCLED WATER

The State of California Water Resources Control Board's Blue Ribbon Panel on Contaminants of Emerging Concern (CEC) acknowledges that they and their advisors (including the California Department of Public Health) are not knowledgeable enough regarding the issue of the safety or lack of safety of the presence of multi-antibiotic resistant organisms in recycled water. The recycled water is, among other things, planned to be indirectly used for: a,) potable water and b.) directly used for irrigation of crops eaten raw as well as pasture lands for livestock eventually marketed for human consumption. The Panel recommends further research by the CDC.

We suggest that this logical conclusion be followed by logical recommendations. Namely, that a temporary moratorium be imposed on recycled water for the following uses: indirect use for potable water systems and direct use for both a.) irrigation of crops eaten raw and b.) pasture lands for livestock eventually marketed for human consumption. The recent presence of resistant pathogens in communities (and not just in hospitals) which are causing life threatening illnesses (initiated by Staph aureus, very dangerous species of E. coli and Klebsiella) are extremely worrisome from a Public Health perspective. If such accelerated presence of antibiotic resistant organisms and their illnesses continue, the safety of many individuals of all ages and the consequent security of our nation will be at much greater risk.

The Panel acknowledges that some uncertainties exist regarding the safety of the presence of multi-antibiotic resistant microbial pathogens. Its conclusion is to encourage additional research by the CDC. This stance by the Panel is confusing for the public as well as decision makers. There is already proof that the multiple barrier concept and the level of disinfection presently utilized by wastewater treatment plants for recycled water does not eliminate the presence of the multi-antibiotic resistant pathogens from the final recycled water product. However, we do have plenty of documentation that all over the U.S. agricultural and hamburger recalls continue because of the acute emergence of: a,) serious illness due to the presence of antibiotic resistant E. coli , b.) life threatening pneumonias initiated by antibiotic resistant Klebsiella and c.) disfiguring and lethal Staph aureus infections.

A critical warning in 1982 by an EPA scientist, Meckes, (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC241834/pdf/aem00183-0119.pdf) was published in a peer reviewed scientific journal regarding the serious potential dangers of antibiotic resistant organisms in byproducts of wastewater treatment plants. That paper does not appear in any of the EPA or CDC websites.

Finally and recently, political pressure has been directed toward veterinarians, physicians and the agricultural/livestock industries to stop the excessive use of antibiotics (which stimulate the mutation of the pathogen's genes to become resistant to the antibiotics). That pressure is an excellent and appropriate preventative start. However, the already existing presence of the resistant pathogens at the end of the wastewater treatment process does not say much for the effectiveness of the multiple barrier concept.

In the Final Report, the Panel notes: "There is no doubt that treatment through wastewater plants reduces the number of pathogenic bacteria (Harwood, Levine et al. 2005; Rijal, Zmuda et al. 2009; Zhang, Marrs et al. 2009) However, there is controversy in the literature as to whether the reduction is sufficient (Harwood, Levine et al. 2005; Chang, Toghrol et al. 2007)) and whether the coliform assays used as surrogates are sufficient (Zhang, Marrs et al. 2009)."

The logical conclusion by the Panel should also be preventative and rather conclude on the need for the above moratorium. This would give sufficient time for the CDC to also address the safety of the presence of the additional source of the pathogenic organisms. This would be logical for the public municipality decision makers and the professionals of the medical, veterinarian, agricultural and livestock communities as opposed to the plan to first use recycled water to indirectly create more potable water and continue to use recycled water to irrigate vegetable/fruit crops and pasture lands for livestock to be marketed for human consumption. Since illness prevention should be our joint objective, we should first wait for the CDC to do the requested research during a moratorium. Let's not put the cart before the horse.

Please read the citations within the US/EPA study and the scientific literature since 1982. From the US/EPA study: "Several researchers have pointed out that wastewater, treated or untreated, is a primary contributor of bacteria to the aquatic ecosystem(12, 16, 17, 20, 27, 29). Studies have been conducted which demonstrate that significant numbers of multiple drug-resistant coliforms occur in rivers (17), bays (9), bathing beaches (28),and coastal canals (13)." Waters contaminated by resistant bacteria, once swallowed, are capable of transferring antibiotic resistance within our bodies to (normally occurring) pathogenic species that exist in our bodies. Infections with antibiotic resistant organisms now kill more Americans than AIDS according to CDC. (Edo, you need to reference this)

In Dr. McGowan's discussions with US/EPA, the CDC, and the Inter Agency Task Force on Antibiotic Resistance, he was told by each that no research is planned to evaluate the consequences of our wastewater treatment plants supplying multi-antibiotic resistant organisms to our environments. US/EPA additionally told McGowan that it has no scientists working on this topic. Thus, we go back to the work done on antibiotic resistance by US/EPA three decades ago, which is cited above, stating:---------------"wastewater, treated or untreated, is a primary contributor of bacteria to the aquatic ecosystem".The Panel notes, within the Final Report, that there are"-------concerns that California drinking water augmentation projects may add to the problem of antibiotic-resistant bacteria (that are) in trace amounts (and) are not likely to be a problem in California water recycling programs." It is, in fact, that the genetic information is the worrisome issue. That seems to be missed on the Panel. The focus of the panel on only trace amounts of antibiotics and antimicrobials, while important to the maintenance of resistance, is not the key issue. It is the ability of genes to transfer to humans the antibiotic resistance. They are so small that they pass through typical filters used in water quality control and are immune to chlorine used at typical levels in water treatment; thus, they survive. In the Final Report, the Panel notes: "The concentrations of these antibiotics and antimicrobials, and others, in finished water that are used for recharge projects are below levels that cause resistance to occur de novo (Watkinson, Murby, et al. 2007) and thus are not likely to be the source of antibiotic resistance." Again, the principal point is the transfer in the human intestine of genetic information from swallowed (already existing) resistant pathogens put into the environment by the wastewater treatment plants. Thus, the above assurance by the Panel may tend to persuade the Board's decision-makers that all is well.

The Panel continues: "At sub-inhibitory doses, antibiotics may lead to increased resistance in bacteria – but the concentrations found in recycled water are at least three orders of magnitude lower than the concentrations needed for resistance (Watkinson, Murby et al. 2007)". Again, the Panel does not understand that the issue is the very small amount of transferred resistant genes to the pathogenic and normally occurring bacteria in our intestines. The latter then act as a lending library for later transfer to pathogens. The above statement by the Panel may lead decision-makers down a primrose path. When the usual scientific knowledge about contagion is discussed, the thinking includes the concentration of the infectious organisms (classically called dose response). However, when thinking about contagion with resistant genes, only a single gene is necessary.

Within the Final Report, the Panel notes: Treatment processes at reclamation facilities effectively reduced the amount of both MRSA and the mecA gene. However, (it) did not eliminate them (Börjesson, Melin et al. 2009). Again, after swallowing a minute number of resistant genes, the transfer of these resistant genes to our normal and pathogenic intestinal bacteria occurs and the subsequent multiplication of the now contaminated intestinal bacteria is extremely rapid People with compromised immune systems are extremely vulnerable to becoming seriously ill and those with well functioning immune systems may become carriers.

The Panel notes that more investigation is warranted. We would agree that this is true and thus the decision-makers (the State Board) must await such investigation before blindly lunging ahead and consequently putting the public at risk. To move ahead absent this critical information would be reckless.

The Panel mentions the work of Harwood which was predicated on the WERF study by Rose that came out in detail in 2004. Let's look at Harwood's work, a study conducted over a year's time which reviewed finished reclaimed (recycled) water in Florida, Arizona and also in California under Title 22: "Microorganisms were detected in disinfected effluent samples at the following frequencies: total coliforms, 63%; fecal coliforms, 27%; enterococci, 27%; C. perfringens, 61%; F-specific coliphages, <http://aem.asm.org/math/sim.gif40%; and enteric viruses, 31%. Cryptosporidium oocysts and Giardia cysts were detected in 70% and 80%, respectively, of reclaimed water samples. Viable Cryptosporidium, based on cell culture infectivity assays, was detected in 20% of the reclaimed water samples. No strong correlation was found for any indicator-pathogen combination. When data for all indicators were tested using discriminant analysis, the presence/absence patterns for Giardia cysts, Cryptosporidium oocysts, infectious Cryptosporidium, and infectious enteric viruses were predicted for over 71% of disinfected effluents. The failure of measurements of single indicator organism to correlate with pathogens suggests that public health is not adequately protected by simple monitoring schemes based on detection of a single indicator, particularly at the detection limits routinely employed." (http://aem.asm.org/cgi/content/short/71/6/3163)

It is quite clear from the work of Harwood that the finished reclaimed (recycled) water is not, by any stretch of the imagination, free of human health risk.Thus, until the State of California has a full grasp of the potential impacts to human health from the use of recycled water and decides how to rectify the potential negative impacts, The Blue Ribbon Panel should place a moratorium on its use for artificial recharge and its use in all types of food production.

1. Breindl M. The structure of heated poliovirus particles. J. General Virology, 1971, vol. 11:147-156. http://vir.sgmjournals.org/cgi/reprint/11/3/147.pdf
2. Grabow, W. O. K., and O. W. Prozesky. Drug resistance of coliform bacteria in hospital and city sewage. Antimicrob. Agents Chemother. 1973 (Feb), Vol. 3:175-180. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC444383/
3. Linton, K. B., M. H. Richmond, R. Bevan, and W. A. Gillespie. Antibiotic resistance and R factors in coliform bacilli isolated from hospital and domestic sewage. J. Med. Microbiol. 1974. 7:91-103. http://jmm.sgmjournals.org/cgi/reprint/7/1/91.pdf
4. Ward RL et al. Inactivation of poliovirus in digested sludge. Appl Environ Microbiol. 1976 June; 31(6): 921–930. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC169857/
5. Gerba CP et al. Effect of sediments on the survival of Ericherichia coli in marine waters. AEM July 1976 114-20. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC170015/

6. Fontaine TD et al. Transferable drug resistance associated with coliforms from hospital and domestic sewage. Health Lab Sci. 1976 Oct; 13(4): 238-45. http://www.ncbi.nlm.nih.gov/pubmed/789292
7. Lance JC et al. Virus movement in soil columns flooded with secondary sewage effluent. AEM Oct 1976 p. 520-26. http://aem.asm.org/cgi/content/short/32/4/520
8. Schaub SA et al, Virus and bacteria removed from wastewater by rapid infiltration through soil. Bacteriophage movement in ground water at distances of 600 feet from site of application. Appl Environ Microbiol. 1977 March; 33(3): 609–619. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC170733/
9. Gerba CO. Poliovirus removal from primary and secondary sewage effluent by soil filtration. AEM Aug 1978 p. 247-51. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC291209/
10. LaBelle RL, et al. Influence of pH, salinity and organic matter on the absorption of enterovirus to estuarine sediments. AEM July 1979 93-101. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC243441/
11. Cenci G, Morozzi G, Daniele R, Scazzocchio F. Antibiotic and metal resistance in "Escherichia coli" strains isolated from the environment and from patients. Ann Sclavo 1980 Mar-Apr;22(2):212-26. http://www.ncbi.nlm.nih.gov/pubmed/7008715
Abstract
The incidence and the patterns of the antibiotic and metal resistance in 106 strains of Escherichia coli isolated from ground waters, used also as drinking water supply (sample A), was studied in comparison with the resistance behaviour in the 104 strains of the same microorganism isolated from non hospitalized patients (sample P). Significant differences between the percentage of resistant strains in the two examined samples were found for some of the antibiotics and the metals tested (ampicillin, streptomycin, kanamycin, mercury and zinc) while non statistically significant differences were found for gentamicin, tetracyclin, nalidixic acid and cadmium. From the high percentages of the resistant strains in the environmental sample (up to 44.3% for tetracyclin) we may deduce that also the ground waters, especially if used as drinking water, contribute to the spread of the resistant bacteria. The patterns of the antibiotic multiresistances in the strains isolated from patients and from ground waters do not differ greatly and this strengthens the hypothesis that resistance to antibiotics has been acquired by Escherichia coli strains before reaching the ground waters.
12. Mark C. Meckes. Effect of UV Light Disinfection on Antibiotic-Resistant, APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1982, p. 371-377 Vol. 43, No. 2. Wastewater Research Division, Municipal Environmental Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268. Received 15 June 1981/Accepted 13 October 1981;

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC241834/pdf/aem00183-0119.pdf
Waters contaminated by bacteria capable of transferring drug resistance are of great concern since there is the potential for transfer of antibiotic resistance to a pathogenic species.

13. Mach PA, et al. R-plasmid transfer in a wastewater treatment plant. AEM 1982 Dec;44(6):1395-403. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC242202/
14. Ribeiro-Dias JC, Vicente AC, Hofer E. Fecal coliforms in sewage waters. I. Resistance to antibiotics, heavy metals and colicinogeny. Appl Environ Microbiol 1983 Jul;46(1):227-32. Others, have found similar results. Mach PA, et al. R-plasmid transfer in a wastewater treatment plant. AEM 1982 Dec;44(6):1395-403. Fontaine TD et al. Transferable drug resistance associated with coliforms from hospital and domentic sewage. Health Lab Sci. 1976 Oct; 13(4): 238-45. Walter, M. V., and J. W. Vennes. 1985. Occurrence of multiple-antibiotic-resistant enteric bacteria in domestic sewage and oxidation lagoons. Appl. Environ. Microbiol. 50:930-933. Rhodes G, Huys G, Swings J, McGann P, Hiney M, Smith P, Pickup RW. Distribution of oxytetracycline resistance plasmids between aeromonads in hospital and aquaculture environments: implication of Tn1721 in dissemination of the tetracycline resistance determinant tet A. Appl Environ Microbiol 2000 Sep;66(9):3883-90. Grol A, Szymanska B, Wejner H, Kazanowski A, Wlodarczyk K. The role of mechanically purified city sewers in the spread of antibiotic-resistant bacteria of the Enterobacteriaceae family] Med Dosw Mikrobiol 1989;41(2):100-5. Lewis GD, et al. Enteroviruses of human origin and faecal coliforms in river water and sediments down stream from a sewage outfall in the Taieri River, Otago. New Zealand Journal of Marine and Freshwater Research, 1986, Vol.20: 101-105. Marcinek H, Wirth R, Muscholl-Silberhorn A, Gauer M. Enterococcus faecalis gene transfer under natural conditions in municipal sewage water treatment plants. Appl Environ Microbiol 1998 Feb;64(2):626-32.
15. Ward RL, et al. Minimum infective dose of animal viruses. Critical Reviews in Environmental Science and Technology, 1984, Vol. 14:278-310. http://www.informaworld.com/smpp/content~content=a907576074~db=all
16. Walter, M. V., and J. W. Vennes. 1985. Occurrence of multiple-antibiotic-resistant enteric bacteria in domestic sewage and oxidation lagoons. Appl. Environ. Microbiol. 50:930-933. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC291771/
17. Walter, M. V., and J. W. Vennes. 1985. Occurrence of multiple-antibiotic-resistant enteric bacteria in domestic sewage and

oxidation lagoons. Appl. Environ. Microbiol. 1985 (Oct), Vol. 50:930-933. http://aem.asm.org/cgi/reprint/50/4/930.pdf

18. Lewis GD, et al. Enteroviruses of human origin and faecal coliforms in river water and sediments down stream from a sewage outfall in the Taieri River, Otago. New Zealand. Journal of Marine and Freshwater Research, 1986, Vol.20: 101-105. http://www.springerlink.com/content/h631n7603v407557/
19. Grol A, Szymanska B, Wejner H, Kazanowski A, Wlodarczyk K. The role of mechanically purified city sewers in the spread of antibiotic-resistant bacteria of the Enterobacteriaceae family] Med Dosw Mikrobiol 1989;41(2):100-5. http://www.ncbi.nlm.nih.gov/pubmed/2586189
20. Nakamura S, Shirota H. Behavior of drug resistant fecal coliforms and R plasmids in a wastewater treatment plant. Nippon Koshu Eisei Zasshi, 1990, Feb;37(2):83-90. http://www.ncbi.nlm.nih.gov/pubmed/2131972
Fecal coliforms were isolated from the inlet, the primary sedimentation tank, the activated sludge digestion tank, the final settling tank, the outlet and the return activated sludge drain at the municipal wastewater plant in Ube City, and examined for drug resistance and presence of R plasmids. Drug concentrations employed to distinguish resistant isolates from sensitive isolates were 25 micrograms/ml for tetracycline, kanamycin, chloramphenicol and streptomycin, 50 micrograms/ml for ampicillin, nalidixic acid and rifampicin, and 200 micrograms/ml for sulfisoxazole, respectively. Of a total of 900 isolates, 45.7% were drug resistant and 51.1% of them carried R plasmids. **The further along that wastewater had progressed through the treatment process the greater the tendency was for appearance of the multiresistant isolates.** These isolates also were shown to simultaneously carry transferable R plasmids. Observed resistant patterns of R plasmids were mainly multiple and encoded to resistance to tetracycline, chloramphenicol, streptomycin and sulfisoxazole. It became clear that multiplication of R plasmids took place in the activated sludge digestion tank. This study show that drug resistance transfer mediated by these R plasmids may occur in actual wastewater treatment plants.
Tracking Antibiotic Resistance Genes (ARG) as Emerging Environmental Contaminants in the Cache La-Poudre River Watershed
21. Abbaszadegan M et al. Detection of of enteroviruses in groundwater with PCR. AEM May 1993 1318-24. http://aem.asm.org/cgi/content/short/59/5/1318
22. Marcinek H, Wirth R, Muscholl-Silberhorn A, Gauer M. Enterococcus faecalis gene transfer under natural conditions in municipal sewage water treatment plants. Appl Environ Microbiol

1998 Feb;64(2):626-32.
http://www.ncbi.nlm.nih.gov/pubmed/9464401

23. Al-Ahmad A, Daschner FD, Kummerer K. Biodegradability of cefotiam, ciprofloxacin, meropenem, penicillin G, and sulfamethoxazole and inhibition of waste water bacteria. Arch Environ Contam Toxicol. 1999 Aug;37(2):158-63. http://www.ncbi.nlm.nih.gov/pubmed/10398765
24. Kummerer K. Drugs, diagnostic agents and disinfectants in wastewater and water--a review. Schriftenr Ver Wasser Boden Lufthyg. 2000;105:59-71. http://www.ncbi.nlm.nih.gov/pubmed/10842795
25. Rhodes G, Huys G, Swings J, McGann P, Hiney M, Smith P, Pickup RW. Distribution of oxytetracycline resistance plasmids between aeromonads in hospital and aquaculture environments: implication of Tn1721 in dissemination of the tetracycline resistance determinant tet A. Appl Environ Microbiol 2000 Sep;66(9):3883-90. http://www.ncbi.nlm.nih.gov/pubmed/10966404
26. National Research Council of the National Academy of Sciences (NAS) Biosolids Applied to Land: Advancing Standards and Practices. Washington, DC: National Academy Press, 2002. http://www.epa.gov/waterscience/biosolids/nas/complete.pdf
27. Heberer T, Reddersen K, Mechlinski A. From municipal sewage to drinking water: fate and removal of pharmaceutical residues in the aquatic environment in urban areas. . Water Sci Technol 2002;46(3):81-8. http://www.ncbi.nlm.nih.gov/pubmed/12227607
28. Seveno, Nadine A. et al. Reviews in Medical Microbiology. Jan 2002, 13(1): 15-27. http://journals.lww.com/revmedmicrobiol/Abstract/2002/01000/Occurrence_and_reservoirs_of_antibiotic_resistance.2.aspx
29. Iversen A, Kuhn I, Franklin A, Mollby R. High prevalence of vancomycin-resistant enterococci in Swedish sewage. Appl Environ Microbiol 2002 Jun;68(6):2838-42. http://aem.asm.org/cgi/content/short/68/6/2838
30. Golet EM et al. Determination of fluoroquinolone antimicrobial agents in sewage sludge and sludge treated soils using accelerated solvent extraction followed by solid phase extraction. Anal Chem. 2002 Nov 1;74(21):5455-62. http://pubs.acs.org/doi/abs/10.1021/ac025762m
31. Robertson GT, Zhao J, Desai BV, Coleman WH, Nicas TI, Gilmour R, Grinius L, Morrison DA, Winkler ME. Vancomycin tolerance induced by erythromycin but not by loss of vncRS, vex3, or pep27 function in Streptococcus pneumoniae. J Bacteriol. 2002 Dec;184(24):6987-7000.]. http://jb.asm.org/cgi/content/short/184/24/6987
32. A. Schlüter, et al.The 64 508 bp IncP-1 antibiotic multiresistance plasmid pB10 isolated from a waste-water treatment plant provides

evidence for recombination between members of different branches of the IncP-1 group. Microbiology 149 (2003), 3139-3153; DOI 10.1099/mic.0.26570-0.
http://mic.sgmjournals.org/cgi/content/abstract/149/11/3139
The complete 64 508 bp nucleotide sequence of the IncP-1 antibiotic-resistance plasmid pB10, which was isolated from a waste-water treatment plant in Germany and mediates resistance against the antimicrobial agents amoxicillin, streptomycin, sulfonamides and tetracycline and against mercury ions, was determined and analysed. It thus appears that plasmid pB10 acquired as many as five resistance genes via three transposons and one integron, which it may rapidly spread among bacterial populations given its high promiscuity…".

33. Amy Pruden. Tracking Antibiotic Resistance Genes (ARG) as Emerging Environmental Contaminants in the Cache La-Poudre River Watershed, Applied and Environmental Microbiology, March 2003, p. 1452-1456, Vol. 69, No. 3. http://aem.asm.org/cgi/content/short/69/3/1452. DNA encoding antibiotic resistance genes (ARG) has recently been established as an emerging contaminant. ARGs have been found in a wide cross-section of environmental compartments, including: wastewater treatment plant effluent, biosolids, agricultural lagoons, groundwater, surface water, and drinking water.
34. Walsch, C. Antibiotics----, Actions, Origins, Resistance, (March 2003) New York: ASM Press.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2286585/
35. Reinthaler FF, Posch J, Feierl G, Wust G, Haas D, Ruckenbauer G, Mascher F, Marth E. Antibiotic resistance of E. coli in sewage and sludge. Water Res 2003 Apr;37(8):1685-90.
http://www.sciencedirect.com/science
36. Hughes D., Department of Cell and Molecular Biology, Box 596, The Biomedical Center, Uppsala University, S-751 24 Uppsala, Sweden. diarmaid.hughes@icm.uu.se, Exploiting genomics, genetics and chemistry to combat antibiotic resistance. Nat Rev Genet. 2003 Jun;4(6):432-41.
We are currently facing a growing number of infections, and a growing number of patients who are dying from infectious diseases. At the same time, the number of reports on drug-resistant strains of bacteria is rising, while industrial efforts in antibiotic discovery and development have been declining.
http://www.ncbi.nlm.nih.gov/pubmed/12776213)
<http://www.ncbi.nlm.nih.gov/pubmed/12776213
37. Cooley MB. Colonization of Arabidopsis thaliana with Salmonella enterica and Enterohemorrhagic Escherichia coli O157:H7 and Competition by Enterobacter asburiae. Applied and Environmental Microbiology, August 2003, p. 4915-4926, Vol. 69, No. 8.
http://aem.asm.org/cgi/content/short/69/8/4915

38. Golet EM, et al. Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. Environ Sci Technol. 2003 Aug 1;37(15):3243-9. http://pubs.acs.org/doi/abs/10.1021/es0264448
39. Kummerer K. Promoting resistance by the emission of antibiotics from hospitals and households into effluent. Clin Microbiol Infect. 2003 Dec;9(12):1203-14. http://www.ncbi.nlm.nih.gov/pubmed/14686985
40. Shivi Selvaratnam and J. David Kunberger. Increased frequency of drug-resistant bacteria and fecal coliforms in an Indiana Creek adjacent to farmland amended with treated sludge. Can. J. Microbiol./Rev. can. microbiol. 50(8): 653-656 (2004). http://rparticle.web-p.cisti.nrc.ca/rparticle/AbstractTemplateServlet?calyLang=eng&journal=cjm&volume=50&year=2004&issue=8&msno=w04-065
41. V. Chitnis, D. Chitnis, S. Patil and Ravi Kant. Hospital effluent: A source of multiple drug-resistant bacteria. Water Research, January 2004, Volume 38, Issue 2, pp 255-494, <http://www.sciencedirect.com/science/journal/00431354>
The present work was carried out to study the spread of multiple drug-resistant (MDR) bacteria from hospital effluent to the municipal sewage system. The MDR bacteria population in hospital effluents ranged from 0.58 to 40% for ten hospitals studied while it was less than 0.00002 to 0.025% for 11 sewage samples from the residential areas. Further, the MDR bacteria carried simultaneous resistance for most of the commonly used antibiotics and obviously the spread of such MDR bacteria to the community is a matter of grave concern.
42. Kummerer K. Standardized tests fail to assess the effects of antibiotics on environmental bacteria. Water Res. 2004 Apr;38(8):2111-6. Kummerer K. Biodegradability of some antibiotics, elimination of the genotoxicity and affection of wastewater bacteria in a simple test. Chemosphere. 2000 Apr;40(7):701-10. http://www.ncbi.nlm.nih.gov/pubmed/15087192
43. Rooklidge SJ. Environmental antimicrobial contamination from terraccumulation and diffuse pollution pathways. Sci Total Environ. 2004 Jun 5;325(1-3):1-13. Review. http://www.ncbi.nlm.nih.gov/pubmed/15144773
44. Kummerer K. Resistance in the environment. J Antimicrob Chemother. 2004 Aug;54(2):311-20. Epub 2004 Jun 23. http://jac.oxfordjournals.org/content/54/2/311.full
45. Shivi Selvaratnam and J. David Kunberger. Increased frequency of drug-resistant bacteria and fecal coliforms in an Indiana Creek adjacent to farmland amended with treated sludge.Can. J. Microbiol./Rev. can. microbiol. 50(8): 653-656 (2004).
Many studies indicate the presence of human pathogens and drug-

resistant bacteria in treated sewage sludge. Since one of the main methods of treated sewage disposal is by application to agricultural land, the presence of these organisms is of concern to human health.

46. Ray JL, Nielsen KM. Experimental methods for assaying natural transformation and inferring horizontal gene transfer. Methods Enzymol. 2005;395:491-520.
http://www.ncbi.nlm.nih.gov/pubmed/15865981
47. Giacometti A, Cirioni O, Kamysz W, Silvestri C, Licci A, D'Amato G, Nadolski P, Riva A, Lukasiak J, Scalise G. In vitro activity and killing effect of uperin 3.6 against gram- positive cocci isolated from immunocompromised patients. Antimicrob Agents Chemother. 2005 Sep;49(9):3933-6.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1195410/
48. Sjolund et al. Persistence of Resistant Staphylococcus epidermidis after Single Course of Clarithromycin(2005) Emerging Infectious Diseases (Vol. 11, # 9, Sept 2005 @ p. 1389 et seq),
http://www.cdc.gov/ncidod/eid/vol11no09/pdfs/05-0124.pdf
Once gene transfer has taken place, the resistant information may be long standing. A 1-week course of clarithromycin selects for macrolide-resistant S.
epidermidis that may persist up to 4 years after treatment. Highly resistant isolates could be detected 1 year after treatment in 4 patients and 4 years after treatment in 3 patients. Analysis showed that highly resistant strains persisted or 4 years, in the absence of further selection pressure, and that both resistant and susceptible strains were present 4 years after treatment. stably resistant populations increase the risk for treatment failure. Second, resistance in the normal microbiota might contribute to increased resistance in higher-grade pathogens by interspecies genetic transfer. Since the population size of the normal microbiota is large, multiple and different resistant variants can develop, which increases the risk for spread to populations of pathogens. Persisting populations of resistant microbiota further enhance transfer risk, especially if the selecting agent is used for treatment. Third, antimicrobial drugs may affect the stability of residential populations. (McGowan's note: As noted in the paper by Meckes, (US/EPA study, circa 1980) transfer of resistance to the human gut occurs where the internal biots becomes bothy resistant and shifted. Sjolund says very much the same thing and adds that multiple and different resistant variants can develop, which increases the risk for spread to populations of pathogens.
49. Aiello AE, Marshall B, Levy SB, Della-Latta P, Lin SX, Larson E. Antibacterial cleaning products and drug resistance. Emerg Infect Dis. 2005 Oct;11(10):1565-70).
http://www.ncbi.nlm.nih.gov/pubmed/16318697

50. Matt Wook Chang. Toxicogenomic Response of ***Staphylococcus aureus*** **to Peracetic Acid.** ***Environ. Sci. Technol.*****, 2006, 40 (16), pp 5124–5131. http://pubs.acs.org/doi/abs/10.1021/es060354b**
51. Sara Firl. The Importance of Municipal Sewage Treatment in the Spread of Antibiotic Resistance. 106th General Meeting of the American Society for Microbiology. May 21-25, 2006, Orlando, Florida. http://www.cura.umn.edu/reporter/06-Fall/LaPara_et_al.pdf
Our study determined that substantial numbers of antibiotic-resistant bacteria were present in municipal wastewater, and that the existing treatment infrastructure did not adequately prevent release of antibiotic-resistant bacteria into the environment. Many of the bacteria found in the wastewater treatment plant and in the plant effluent were tentatively identified as potential pathogens and were also resistant to multiple antibiotics, raising public health concerns.
52. MJ Higgins, S Murthy. Examination of Reactivation and Regrowth of Fecal Coliforms in Anaerobically Digested Sludge WERF Report: Biosolids and Residuals (03-CTS-13T), 2006 (June).
http://www.werf.org/AM/Template.cfm?Section=Biosolids&CONTENTID=12522&TEMPLATE=/CM/ContentDisplay.cfm
53. Matt Wook Chang. Toxicogenomic response to chlorination includes induction of major virulence genes in Staphylococcus aureus. Env Sci & Tech 2007, vol. 41,no. 21, pp. 7570-7575
Microbial control in aqueous environments entails widespread use of chlorination. Hypochlorous acid induced transcription of genes encoding major virulence factors of S. aureus, such as exotoxins, hemolysins, leukocidins, coagulases, and surface adhesion proteins, which all play essential roles in staphylococcal virulence. This work implies that chlorination may stimulate production of virulence factors, which provides new insight into host-pathogen interactions and effects of chlorine application for microbial control.
54. HJ Jang. *Toxicogenomic* Response of Staphylococcus aureus to Triclosan. The *FASEB Journal.* 2008;22:1023.1.
http://www.fasebj.org/cgi/content/meeting_abstract/22/1_MeetingAbstracts/1023.1
55. Heather Storteboom. et.al Tracking Antibiotic Resistance Genes in the South Platte River Basin Using Molecular Signatures of Urban, Agricultural, And Pristine Sources. Oct 21, 2010.
http://pubs.acs.org/doi/abs/10.1021/es101657s
Antibiotic resistance genes (ARGs) have been recognized by the scientific community as emerging contaminants, independent of their host bacterial cells (1, 2). ARGs are broadly distributed across various environmental matrices (e.g., soils, groundwater, surface water, and sediments) with strong evidence of human activity driving amplification above background occurrence (1-6). Nonnative bacteria possessing ARGs are transported to surface waters via point-sources, such as wastewater treatment plant (WWTP) effluents or runoff from animal feeding operations (AFOs), or

nonpoint sources, such as runoff from agricultural fields where manure or sewage sludge have been applied.